Parental and endosymbiont effects on sex determination in haplodiploid wasps

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English summary

Sex determination and sex differentiation

The separation of and sexual differentiation into female and male sexes is a widespread phenomenon in the animal kingdom. Each of the two sexes produces haploid gametes of which the nuclei fuse in the fertilized egg to yield start the development of a male or female diploid embryo. This process must be of high fidelity, as intersexes usually have a lower fitness. As the individual matures, differentiation of sexual organs and secondary sexual traits occurs. This eventually yields a mature female with ovaries or a male with testes.

Both parents can control the action of genes by activating or silencing them on their provided chromosome sets. Only the mother provides the cytoplasm of the offspring and is able to deposit gene transcripts into her oocytes. This seemingly leaves the male with fewer options to control his offspring, but also he may be able to provide additional information, e.g. by imprinting genes or providing short RNA molecules through the sperm. After fertilization, the embryo starts its development in the presence of a maternal and paternal chromosome set and egg cytoplasm containing parentally derived products. These products in the egg degenerate over time while zygotic transcription starts.

Chromosomal and haplodiploid sex determination

Sex determination systems based on the presence of sex chromosomes are particularly common. In these systems a particular chromosome, containing a sex determining locus, determines the sex of the offspring. In male heterogametic systems all males have both an X and a Y chromosome, whereas females carry a homozygous combination of two X chromosomes. In supplying either an X or a Y chromosome to their spermatocytes, males determine at fertilization whether the embryo will become female or male. Conversely, in female heterogametic systems, the females have a Z and a W chromosome, whereas the males have two Z chromosomes. In these systems females determine the sex of the offspring through their oocytes with either a Z or a W chromosome. Variations to these heterogametic systems occur when either the male or the female lacks the minor sex chromosome, i.e. they have an XO or ZO genotype. In such systems the heterogametic sex determines the sex of the offspring alike the XY and ZW systems by providing either a sex chromosome or not.

Haplodiploid sex determination is a markedly different system, though it is also based on chromosomal configurations of the embryo. Haplodiploid species consist of haploid males and diploid females. Females produce haploid oocytes that do not need to be fertilized to develop into an adult individual. Unfertilized eggs develop into haploid males, whereas fertilized eggs develop into diploid females. The two sexes do not differ for a specific sex chromosome, but in the number of copies of the whole complement. Males only inherit a chromosome set from their mother and subsequently pass this on to their daughters. It means that there is no sex determining locus on a specific sex chromosome as any chromosome may occur in both sexes. Haplodiploidy allows behavioural control over fertilization as females can facultatively allow a sperm to her egg upon oviposition and they can thus control the sex of the offspring.

Yet, in sexually reproducing haplodiploids, the inclusion of a paternal chromosome set is, bar some exceptional cases, the only genetic option to generate a female.

Haplodiploid mechanisms of sex determination

One genetic mechanism of haplodiploid sex determination consists of a paternal genome and maternally provided elements activating the female-specific pathway upon zygotic transcription in the embryo. A hallmark of insect sex determination is the sex-specific splicing that occurs upon the start of female or male development. The molecular genetics of haplodiploid sex determination have thus far only been studied in two species of Hymenoptera: the honeybee (*Apis mellifera*) and the jewel wasp (*Nasonia vitripennis*). These mechanisms are very different in genetic versus epigenetic components and this variation gave the first indication of the fast evolution of sex determination mechanisms in the Hymenoptera.

The honeybee possesses a mechanism of Complementary Sex Determination (CSD), where heterozygosity at one or multiple loci determines the sex of the offspring. Heterozygous diploid individuals will develop into females, whereas hemizygous haploid individuals or homozygous diploid individuals will become males. This type of mechanism can easily be detected by inbreeding that will increase the level of homozygosity, and hence the ratio of male to female offspring. CSD has been described for many species in many different lineages of the Hymenoptera. The precise molecular mechanism has only been identified in the honeybee. The *csd* gene is a duplication of the key sex determination gene *feminizer* (*fem*), an ortholog of *transformer* (*tra*). Only a heterozygotic state of the *csd* gene can enforce female-specific splicing of the *fem* gene and start the path to female differentiation.

The molecular mechanism of sex determination in *N. vitripennis* is based on two very different principles. Maternal provision of female-specific *tra* transcripts is required to start female-specific splicing of this gene in the embryo. This alone is however not enough to start female development. An element which is silenced in the maternal genome, yet active in the paternal genome, is needed in combination with the female-specific *tra* provision to enable female-specific splicing of *tra*. This imprinting element has not yet been identified, but is not *tra* itself.

The insect sex determination gene cascade

A major question in evolutionary biology is how sex determination pathways evolve. Which genes are involved, how are they regulated, and do these genes have other functions as well? *Tra* is not only a key gene in the sex determination pathway of *A. mellifera* and *N. vitripennis*, but plays a pivotal role in development of all insect species in which its orthologs have been found. Only the female-specific splice variant of *tra* can be translated into a functional protein. This TRA protein forms a complex with TRA2 protein, encoded by the *tra2* gene that does not possess any splice forms restricted to a particular sex and is more conserved than other genes in the sex determination cascade. *Tra2* also has other developmental functions. The combination of TRA2 and TRA ensures that mRNA transcribed from the downstream gene *doublesex* (*dsx*) is spliced in the female mode. DSX is a transcription factor and the female-specific protein will induce female differentiation, while the male-specific protein leads to male differentiation. In *A. mellifera* and *N. vitripennis* the zygotic state of *tra* is in the male mode. A feminizing primary signal is needed to initiate female-specific zygotic transcription of *tra* to start female development. The primary signals, which control this switch of the *tra-dsx* axis, are highly diverged, which hampers identification based on homology.

Dsx has thus far been detected in all investigated insect orders (Chapter 2). This conservation is confirmed in this thesis by its conserved sex-specific transcripts in *Asobara tabida* sexual development (Chapter 4). Its function as the ultimate switch leading to sexual differentiation may be very specific and not easily taken over by another gene. This specificity may not be the case for *tra*, as this gene appears to be absent (or evades detection) in multiple branches of insects (Chapter 2). *Tra* seems to have been lost multiple times from the sex determination cascade or, alternatively, may have been added multiple times in different groups. *Tra* has been duplicated in several families of the Hymenoptera, including non-CSD species, as paralogs were observed in *Leptopilina* wasps (Chapter 2 and 5). Yet it is unclear whether these duplications are involved in sex determination, be it as a CSD locus or as a maternal effect factor.

Maternal provision in sexually reproducing wasps

Maternal provision of both *tra2* (Chapter 3) and female-specific *tra* was found to be required to start female development in *N. vitripennis*. Inclusion of *tra2* mRNA into the egg is a conserved mode of gene regulation in sex determination. Decreased levels of *tra2* mRNA in embryos resulted in diploid male development, but also demonstrated a strong mortality in early life stages. These results indicate that early presence of *tra2* transcripts in the embryo also has other developmental functions in haploid embryos. Furthermore I observed that diploid embryos with a paternal chromosome set have a reduced offspring number if either *tra2* or *tra* is absent in early life stages.

Whereas female-specific *tra* maternal provisioning is crucial for *N. vitripennis*, it was not found in the wasp *A. tabida* (Chapter 4). *A. tabida* females do provide alternatively spliced variants of *tra* to their eggs that code for a shorter protein, which is, however, unlike the male form. The lack of female-specific *tra* in the early stages of diploid female development leaves the question how feminizing activity would be started. The alternative splice forms of *tra* could play a role in activating female-specific transcription of *tra* in the early embryo. An active paternal factor would be required to distinguish between haploid and diploid development. The crucial role of *tra2* maternal provision does appear conserved in *A. tabida* sex determination, as I could detect its presence in embryos prior to zygotic transcription and upon zygotic activation of the sex determination cascade.

Endosymbiont interference with sex determination

In insects whose sex determination mechanism is based on a zygotic state of *tra* in the male mode, a cue for female development is required that switches the sex determination cascade into the female mode. This cue does not necessarily come from the insect itself. Endosymbionts are widespread in many insect orders and can have drastic effects on the reproductive mode of their hosts. Endosymbionts are only transmitted via cytoplasm and, because of this one-sided transmission, benefit from turning genetic male embryos into females or excluding male embryos and switching the progeny sex ratio toward more females. In haplodiploid species they can induce asexual reproduction, meaning that infected diploid females produce infected haploid oocytes that are diploidized and feminized to develop into females.

The parasitoid wasps *Asobara japonica* and *Leptopilina clavipes* consist of both sexual and asexual populations. Asexual wasps of both species are infected with *Wolbachia* endosymbionts. At least for *A. japonica* it is documented that this feminization occurs as a separate step after the diploidization action. The *Wolbachia* infection can be removed with antibiotics treatments which results in the production of haploid male wasps.

Sexual females of *A. japonica* and *L. clavipes* do not provide female-specific *tra* transcripts to their oocytes (Chapter 5 and 6). This phenomenon was found to be shared with the sexual females of *A. tabida* (Chapter 4). Asexual females of both *A. japonica* and *L. clavipes*, however, do add female-specific *tra* transcripts to their oocytes in the presence of *Wolbachia*. When I removed *Wolbachia* from asexual *A. japonica* females, they did not provide the female-specific *tra* transcripts anymore and reverted to the pattern of *tra* provision observed in the sexual females (Chapter 6). Hence, *Wolbachia* appears to have taken over control of sex determination by manipulating the maternal provision of the feminizing *tra*.

Parental and endosymbiont effects on sex determination – who is in control?

Maternal provision and the sex-specific transcripts of sex determination genes during early stages of development yield valuable information for uncovering the molecular mechanisms of sex determination. Female-specific *tra* was found to not be required at the onset of female development under sexual reproduction of three wasp species (*A. tabida*, *A. japonica* and *L. clavipes*) (Chapter 4, 5 and 6). Yet the requirement of *tra2* maternal provision for female development is likely a widespread phenomenon. Additional maternal elements, other than *tra2*, may be required to induce the female-specific pathway, in example the alternative splice variants of *tra* or its paralogs. However, this maternal provision alone is not sufficient to start female development in sexually reproducing wasps. The paternal genome would require an epigenetic difference from the maternal genome to start female-specific zygotic transcription in diploid fertilized eggs. This paternal dependency may hypothetically also be manipulated by *Wolbachia* mimicking the activity of the paternal genome. Alternatively, rather than a distinction between two chromosome sets, the dosage effects resulting from the diploid state may initiate female development. The interference of endosymbionts with the splicing and maternal provision of *tra* suggests the existence of an arms race between maternal and endosymbiotic elements over haplodiploid sex determination. This warrants the question: who is in control of sex determination, and who could take over control of sex determination?

Dutch summary

Geslachtsbepaling en geslachtsdifferentiatie

De scheiding in verschillende geslachten en de differentiatie tot mannelijke en vrouwelijke individuen is een wijdverspreid verschijnsel in het dierenrijk. Beide geslachten produceren haploïde gameten. De ontwikkeling van een mannelijk of vrouwelijk diploïd embryo start als de kernen van deze gameten fuseren in de bevruchte eicel. Deze tweedeling in ontwikkeling moet gewaarborgd zijn, aangezien interseksuele individuen vaak een lagere fitness hebben. Differentiatie van geslachtsorganen en secundaire geslachtskenmerken treedt op gedurende de verdere ontwikkeling. Dit leidt uiteindelijk tot een volgroeid vrouwelijk individu met ovaria of een volgroeid mannelijk individu met testes.

Beide ouders kunnen de activiteit van genen controleren door deze te aan- of uit te zetten op de set chromosomen die zij aan het embryo doorgeven. Alleen de moeder geeft haar cytoplasma mee aan haar nakomelingen en kan hier gen-transcripten in meegeven. Dit lijkt de vader minder mogelijkheden te geven om de ontwikkeling van zijn nageslacht te beïnvloeden. Maar ook hij kan mogelijkerwijs extra elementen meegeven, bijvoorbeeld door genen in te prenten of door korte RNA moleculen met de spermatozoa mee te leveren. Na de bevruchting zal het embryo de eigen ontwikkeling beginnen in aanwezigheid van zowel een maternale als een paternale chromosoomset en een cytoplasma met daarin producten van beide ouders. Deze producten degenereren langzaam terwijl de transcriptie vanaf het genoom de zygote zelf begint.

Chromosomale en haplodiploïde geslachtsbepaling

Geslachtsbepalingssystemen gebaseerd op geslachtschromosomen zijn zeer algemeen. Een bepaald chromosoom in deze systemen bevat een geslachtsbepalingslocus en dit bepaalt, zoals de naam al zegt, het geslacht van de nakomelingen. In mannelijk heterogametische systemen hebben alle mannelijke individuen een X- en een Y-chromosoom, terwijl de vrouwelijke individuen twee X-chromosomen bezitten. Doordat de mannelijke individuen zowel een X- als een Y-chromosoom kunnen meegeven in hun spermatocyten wordt hierdoor bij de bevruchting bepaald of het embryo mannelijk of vrouwelijk wordt. Omgekeerd hebben vrouwelijke individuen in vrouwelijk heterogametische systemen een Z- als een W-chromosoom en de mannelijke individuen twee Z-chromosomen. De oöcyten van de vrouwelijke individuen bepalen in deze systemen het geslacht van de nakomelingen door een Z-chromosoom of een W-chromosoom mee te geven. Er zijn variaties op deze heterogametische systemen, waarbij of het mannelijke type of het vrouwelijke type een geslachtschromosoom mist. In dit geval is er sprake van een XO- of een ZO-genotype..

HaplodiploÏde geslachtsbepaling werkt volgens een ander principe, maar is ook gebaseerd op de chromosomale samenstelling in het embryo. Haplodiploïde soorten bestaan uit haploïde mannelijke individuen en diploïde vrouwelijke individuen. Deze vrouwelijke individuen produceren haploïde oöcyten die niet bevrucht hoeven te worden om te ontwikkelen tot een volwassen nakomeling. Onbevruchte eieren ontwikkelen zich tot haploïde mannelijke individuen, terwijl bevruchte eieren zich ontwikkelen tot diploïde vrouwelijke individuen. De twee geslachten zijn niet gescheiden door een specifiek geslachtschromosoom, maar verschillen in de hoeveelheid chromosoomsets. Mannelijke individuen krijgen alleen een chromosoomset van hun moeder en geven deze vervolgens alleen door aan dochters, want bij haplodiploïden produceren mannen geen zonen. Dit betekent, dat er geen geslachtsbepalingslocus op een specifiek geslachtschromosoom aanwezig is aangezien elk chromosoom in elk geslacht kan voorkomen. Haplodiploïde geslachtsbepaling wordt gecontroleerd door de moeder, die kan kiezen haar eieren te laten bevruchten door ontvangen spermatozoa, en hiermee het geslacht van haar nakomelingen kan bepalen. Eigenlijk is in seksueel voortplantende haplodiploïden, een enkele uitzondering daargelaten, de toevoeging van een paternaal genoom de enige optie om een vrouwelijk individu te verkrijgen.

HaplodiploÏde mechanismen van geslachtsbepaling

Het genetische mechanisme van haplodiploïde geslachtsbepaling is gebaseerd op een paternaal genoom en maternaal toegevoegde elementen waardoor vrouwelijke ontwikkeling tijdens de start van zygotische transcriptie in het embryo in gang wordt gezet. Het belangrijkste kenmerk van geslachtsbepaling in insecten is de geslachtsspecifieke splicing die optreedt bij de start van vrouwelijke en mannelijke ontwikkeling. De moleculaire genetica van haplodiploïde geslachtsbepaling is tot dusver slechts bestudeerd in twee soorten Hymenoptera: de honingbij (*Apis mellifera*) en de juweelwesp (*Nasonia vitripennis*). Deze mechanismen verschillen tussen genetische en epigenetische componenten en deze variatie gaf de eerste aanwijzing voor de snelle evolutie van geslachtsbepalingsmechanismen in de Hymenoptera.

De honingbij bezit Complementaire Sex Determinatie (CSD), waarbij heterozygotie op een of meerdere loci het geslacht van de nakomelingen bepaalt. Heterozygote diploïde individuen ontwikkelen zich vrouwelijk, terwijl hemizygote haploïde individuen en homozygote diploïde individuen uiteindelijk mannelijk worden. Dit type mechanisme kan gedetecteerd worden door inteeltstudies, die het niveau van homozygotie verhogen, en daarmee de ratio mannelijke ten opzicht van vrouwelijke ontwikkeling zullen verhogen. CSD-mechanismen zijn gevonden in veel soorten uit verschillende families van de Hymenoptera. Het precieze moleculaire mechanisme is echter alleen opgehelderd voor in de honingbij. Het *csd* gen is hier een duplicatie van het belangrijke geslachtsbepalingsgen *feminizer* (*fem*), een ortholoog van *transformer* (*tra*). Alleen een heterozygote staat van het *csd* gen kan ervoor zorgen dat *fem*-transcripten op een vrouwelijk specifieke manier geproduceerd worden en hierdeer start vrouwelijke differentiatie.

Het moleculaire mechanisme van geslachtsbepaling in *N. vitripennis* is gebaseerd op twee andere principes. Maternale provisie van vrouwelijk specifieke *tra*-transcripten is nodig om de vrouwelijke transcriptievorm van dit gen te starten in het embryo. Dit alleen is niet afdoende om vrouwelijke ontwikkeling te activeren. Een element, dat geïnactiveerd is op het maternale genoom, maar actief is op het paternale genoom, is nodig in combinatie met vrouwelijk specifieke *tra*-provisie om de vrouwelijk specifieke zygotische transcriptie van *tra* te starten. Het geïnactiveerde element is nog niet geïdentificeerd, maar het is niet *tra* zelf.

De geslachtsbepaling gen-cascade van insecten

Een belangrijke vraag in de evolutionaire biologie is hoe geslachtsbepalingsroutes evolueren. Welke genen zijn hierbij betrokken; hoe worden zij gereguleerd; en hebben deze genen ook andere functies? *Tra* is niet alleen een belangrijk gen in de geslachtsbepalingsmechanismen van *A. mellifera* en *N. vitripennis*, maar speelt een centrale rol in de ontwikkeling van alle insectensoorten, waarin orthologen zijn gedetecteerd. Alleen de vrouwelijke splice variant van *tra* wordt vertaald in een functioneel eiwit. Dit TRA-eiwit vormt een complex met het TRA2-eiwit, dat gecodeerd wordt door het *tra2* gen. *Tra2* bezit geen geslachtsspecifieke splice varianten en is meer geconserveerd dan andere genen in de geslachtsbepalingscascade. Verder heeft het ook nog andere functies in vroege ontwikkeling. De combinatie van TRA en TRA2 zorgt ervoor, dat het mRNA van het volgende gen in de cascade, *doublesex* (*dsx*), wordt gespliced in de vrouwelijke modus. DSX is een transcriptie factor, en het vrouwelijk specifieke eiwit induceert vrouwelijke differentiatie, terwijl het mannelijke eiwit leidt tot mannelijke differentiatie. De zygotische staat van *tra* is in de mannelijke modus in *A. mellifera* en *N. vitripennis*. Een feminiserend primair signaal is nodig om vrouwelijk specifieke zygotische transcriptie van *tra* te initiëren om vrouwelijke ontwikkeling te starten. De primaire signalen, die deze switch van de *tra*-*dsx*-as controleren, zijn sterk gedivergeerd, wat hun identificatie gebaseerd op homologie bemoeilijkt.

Dsx is tot dusver gedetecteerd in alle bestudeerde insecten-ordes (Hoofdstuk 2). Deze conservering wordt bevestigd in deze thesis door de geconserveerde geslachtsspecifieke transcripten in *Asobara tabida* vrouwelijke en mannelijke ontwikkeling (Hoofdstuk 4). De functie van *dsx* als de laatste switch leidend tot seksuele differentiatie is mogelijk zeer specifiek en niet makkelijk over te nemen door een ander gen. Deze functionele conservering is mogelijk niet het geval voor het *tra* gen, aangezien dit gen afwezig lijkt te zijn (of niet te detecteren is) in verschillende groepen insecten (Hoofdstuk 2). *Tra* lijkt meermaals verloren te zijn gegaan uit de geslachtsbepalingscascade of, in een alternatief scenario, meermaals te zijn toegevoegd aan de geslachtsbepalingscascade. *Tra* is gedupliceerd in verschillende families van de Hymenoptera, ook in soorten zonder CSD, aangezien ik paralogen heb gevonden in *Leptopilina* wespen (Hoofdstuk 2 en 5). Het is echter nog onduidelijk of de wijdverbreide duplicaties betrokken zijn bij geslachtsbepaling, of dat nu is als een CSD locus of als een factor met maternale effecten.

Maternale provisie in seksueel voortplantende wespen

Maternale provisie van zowel *tra2* (Hoofdstuk 3) en vrouwelijk specifieke *tra* is nodig voor de start van vrouwelijke ontwikkeling in *N. vitripennis*. De toevoeging van *tra2* mRNA in de eieren is een geconserveerd element van gen-regulatie in geslachtsbepaling. Verminderde hoeveelheden *tra2* mRNA in embryo's resulteerde in de ontwikkeling van diploïde mannelijke individuen in *N.*

vitripennis (Hoofdstuk 3). Verder was er ook een sterke vermindering van hoeveelheid nakomelingen te zien. Dit geeft aan dat de vroege aanwezigheid van *tra2*-transcripten in het embryo ook andere functies in de ontwikkeling van haploïde embryo's heeft. Daarnaast observeerde ik een sterkere daling in het aantal diploïde nakomelingen met een paternale chromosoomset na vermindering van zowel *tra*- als *tra2*-transcipten in vroege stadia van ontwikkeling.

Ondanks dat vrouwelijk specifieke *tra*-transcripten cruciaal zijn tijdens de vroege ontwikkeling van *N. vitripennis* en maternaal meegeleverd worden, is dit niet het geval in de wesp *A. tabida* (Hoofdstuk 4). In plaats daarvan geven *A. tabida* moeders alternatieve splice varianten van *tra* mee aan hun eieren. Deze coderen voor een korter eiwit, dat echter een belangrijk domein bevat en niet zo kort is als de mannelijk specifieke eiwitten. Het gebrek aan vrouwelijk specifieke *tra* in de vroege stadia van diploïd vrouwelijke ontwikkeling opent echter de vraag hoe de vrouwelijk activiteit gestart wordt in dit systeem. De alternatieve splice vormen van *tra* kunnen mogelijk een rol spelen in de activatie van de vrouwelijk specifieke transcriptie van *tra* in het vroege embryo. Daarnaast zou echter een factor nodig zijn om het verschil tussen haploïde en diploïde ontwikkeling aan te geven, mogelijk een actieve paternale factor of een herkenning van dosis tussen een enkel of een dubbel genoom. De cruciale rol van *tra2* maternale provisie lijkt wel geconserveerd te zijn in *A. tabida* geslachtsbepaling. Deze transcripten kon ik detecteren in embryos voorafgaand aan zygotische transcriptie en tijdens de zygotische activatie van de geslachtsbepalingscascade (Hoofdstuk 4).

Verstoring van geslachtsbepaling door endosymbionten

Bij insecten waarvan het geslachtsbepalingsmechanisme is gebaseerd op een zygotische staat van *tra* in de mannelijke modus, is een aanzet voor vrouwelijke ontwikkeling is nodig die de geslachtsbepalingscascade in de vrouwelijke modus plaatst. Deze aanzet hoeft niet perse vanuit het insect zelf te komen. Endosymbionten zijn wijdverspreid in veel insect-ordes en kunnen drastische effecten hebben op de voortplanting van hun gastheren. Endosymbionten worden alleen doorgegeven via het cytoplasma. Vanwege deze eenzijdige transmissie is het in hun voordeel om genetisch mannelijke embryo's op een pad van vrouwelijke ontwikkeling te zetten of om deze mannelijke embryo's te verwijderen en het percentage vrouwelijke nakomelingen hoger te maken. In haplodiploÏde soorten kunnen endosymbionten ervoor zorgen, dat de gastheren zich aseksueel voortplanten. Geïnfecteerde diploïde moeders leggen geïnfecteerde haploïde eieren, die diploïd gemaakt en gefeminiseerd worden tot een volgende generatie van geïnfecteerde diploïde vrouwelijke individuen.

De parasitaire wespen *Asobara japonica* en *Leptopilina clavipes* hebben elk seksueel en aseksueel voortplantende populaties. Aseksuele wespen van beide soorten zijn geïnfecteerd door *Wolbachia* endosymbionten. Voor *A. japonica* is gedocumenteerd, dat de feminisering een aparte stap na de diploïdisatie is. De *Wolbachia* infectie kan verwijderd worden met antibiotica-behandelingen, die resulteren in de productie van haploïde mannelijke wespen.

Seksueel voortplantende *A. japonica* en *L. clavipes* wespen geven geen vrouwelijk specifieke *tra*-transcripten mee aan hun oöcyten (Hoofdstuk 5 en 6). Dit fenomeen komt overeen met het patroon van *tra*-transcripten, dat ik detecteerde in de seksuele voortplanting van *A. tabida* (Hoofdstuk 4). Aseksuele *A. japonica* en *L. clavipes* wespen geven echter wel vrouwelijk specifieke *tra*-transcipten mee aan hun oöcyten in de aanwezigheid van *Wolbachia*. Wanneer ik *Wolbachia* verwijderde uit de aseksuele *A. japonica* wespen, gaven zij geen vrouwelijk specifieke *tra*-transcripten meer mee, maar waren ze in plaats daarvan terug gegaan naar het patroon van *tra*-transcripten dat wordt meegegeven in seksuele wespen (Hoofdstuk 6). Hieruit concluderend lijkt *Wolbachia* de controle over geslachtsbepaling te hebben overgenomen door de maternale provisie van vrouwelijk specifieke *tra*-transcripten te manipuleren.

Ouderlijke en endosymbiont effecten op geslachtsbepaling – wie heeft de controle?

Maternale provisie en de geslachtsspecieke transcripten van geslachtsbepalingsgenen gedurende vroege stadia van ontwikkeling bieden kostbare informatie aangaande de nog te ontdekken moleculaire mechanismen van geslachtsbepaling. Aanwezigheid van vrouwelijk specifiek *tra* is niet noodzakelijk voor vrouwelijke ontwikkeling in drie seksueel voortplantende wespen soorten (*A. tabida*, *A. japonica* en *L. clavipes*) (Hoofdstuk 4, 5 en 6). Maternale provisie van *tra2* lijkt daarentegen wijdverbreid noodzakelijk te zijn voor vrouwelijke ontwikkeling. Andere maternale elementen, bijvoorbeeld alternatieve splice varianten van *tra* of paralogen daarvan, kunnen ook vereist zijn voor het starten van de vrouwelijke geslachtsbepalingscascade. Echter, alleen maternale provisie is niet afdoende om vrouwelijke ontwikkeling te starten in seksueel voortplantende wespen. Het paternale genome zou een epigenetisch verschil met het maternale genoom moeten bezitten om vrouwelijk specifieke transcriptie te starten in diploïde bevruchte eieren. Deze afhankelijkheid van de chromosoom set van de vader kan hypothetisch ook gemanipuleerd worden door *Wolbachia*, waarbij de endosymbiont de activiteit van het paternale genoom kan nabootsen. Alternatief kan het, in plaats van een verschil tussen het maternale en paternale genoom, echter ook het dosis effect van de diploïde staat van het embryo zijn die herkend wordt en vrouwelijke ontwikkeling start. De verstoring van *tra* maternale provisie suggereert, dat gastheer en endosymbiont elkaar proberen te overheersen in een conflict over de controle van haplodiploïde geslachtsbepaling. Dit roept de vraag op: wie heeft zeggenschap over geslachtsbepaling, en wie zou het beheer kunnen overnemen?

Curriculum vitae

Elzemiek Geuverink was born on the 15th of February 1985 in Enschede, The Netherlands. She started her BSc Biology at the University of Groningen in 2003 and graduated in 2006 with a specialization in Ecology. In 2006 she continued with her MSc Ecology studies. Her MSc research included a project on gametic isolation in *Nasonia* wasps, a study on above- and belowground interactions in a salt marsh ecosystem and the implementation of in situ hybridization techniques in *Nasonia*. She graduated with honours (*cum laude*) in 2008 and started as an Early Stage Researcher at the University of Jyväskylä (Finland) within the Marie Curie Initial Training Network "Speciation". In Jyväskylä she studied the variation in developmental mode and reproduction in *Pygospio* worms (at the University of Jyväskylä) and *Littorina* snails (at the University of Sheffield). In 2010 she started the PhD project presented in this thesis at the University of Groningen. During 2014-2015 she worked as a teaching assistant, and currently she is working as a researcher in the Evolutionary Genetics, Development & Behaviour cluster at the University of Groningen studying the mechanisms with which *Wolbachia* interferes with sex determination in parasitoid wasps.

List of publications

Geuverink E & Beukeboom LW (2014) Phylogenetic distribution and evolutionary dynamics of the sex determination genes *doublesex* and *transformer* in insects. *Sexual Development*, **8**, 38-49

Kesäniemi JE, Geuverink E & Knott KE (2012) Polymorphism in developmental mode and its effect on population genetic structure of a spionid polychaete, *Pygospio elegans*. *Integrative and Comparative Biology*, **52**, 181-196

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Veen GF, Geuverink E & Olff H (2012) Large grazers modify effects of aboveground-belowground interactions on small-scale plant community composition. *Oecologia*, **168** , 511-518

Geuverink E, Gerritsma S, Pannebakker BA & Beukeboom LW (2009) A role for sexual conflict in the evolution of reproductive traits in *Nasonia* wasps? *Animal Biology,* **59**, 417-434

Submitted:

Geuverink E, Rensink AH, Rondeel I, Beukeboom LW, Van de Zande L & Verhulst EC (submitted) Maternal provision of *transformer-2* is required for female development and embryo viability in *Nasonia vitripennis*

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